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RESEARCH ARTICLE

Screening of Neuroprotective Action of Hydroethanolic Extract of Leaf of *Clematis buchananiana* in Diabetic-Induced Neuropathy

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Abstract

Diabetic neuropathy is the most dangerous complication of diabetes which is very difficult to treat. Diagnosis in the early stage prevents worse outcomes of the illness. Determine the pharmacological effect of hydroethanolic extract of *Clematis buchananiana* leaves was investigated and screened for determination of neuroprotective effect in diabetic-induced neuropathy using Wistar rats. Streptozotocin normally induces diabetes within 3 days. The destruction of the beta cells present in the pancreatic gland leads to diabetes. The confirmation of diabetes was done with the help of a glucometer. The experimental models used for the assessment of analgesic activity in Wistar albino rats included the tail immersion method and acetic acid-induced writhing method. After completion of the research study, it was found that the animals treated with standard drug (Gabapentin, 100 mg/kg) had maximum analgesic action, followed by a higher dose of hydroethanolic extract of *Clematis buchananiana* leaf (HEELCB, 400 mg/kg). The major observation was that hydroethanolic extract of *Clematis buchananiana* has significant analgesic action and it validates the traditional claim of the plant as an analgesic agent.

Keywords: Beta Cells, Hyperalgesia, Hyperglycemia, Neuropathic Pain, Streptozotocin, Tail-Flick Latency

1. Introduction

The survival of humans had been always a challenging task since ancient times. History tells us that a small insect to a larger animal or even an invisible micro-organism can lead to mortality. But, thanks to researchers and scientists who worked hard to develop vaccines, equipment, and all necessary health accessories which had helped for survival for humans to date[1]. The vaccination of various pathological organisms no doubt had increased the immunity of the human race but it cannot be denied by the fact that resistance in viruses and bacteria had also grown on the other hand[2]. Similarly, the lifestyle disorder diabetes had also emerged as one of the most common diseases which is very common that is one in every 10 people. Diabetes can be defined as the body's inability to maintain the level of blood glucose in the body[3]. The common symptoms of diabetes include hyperglycemia, weight loss, polyphagia, polydipsia, fatigue, weakness, pain, nausea etc[4]. The causes may be less secretion of insulin in the body, or other factors like the delayed release of insulin, decreased receptor sensitivity, modification in active binding sites of the active drug moiety, mutation, ageing etc[5]. Diabetes along with slow progression and ignorance can lead to the development of serious complications like renal failure, retinopathy, neuropathy etc. Among these diabetic neuropathies have emerged as one of the most unnoticed complications of diabetes which affects fifty per cent population who are

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affected by diabetes[6]. Diabetic neuropathy is a state in which the nerves are damaged due to hyperglycemia in the progression of diabetes. The situation gets worse if untreated as it can affect the various nerves throughout the body. The more common areas include the leg and feet[7]. The which are commonly observed in diabetic neuropathy include pain and numbness in the feet and legs, nausea, and weakness. In severe cases, tingling pain with burning sensations can be felt which starts from in the toes and fingers progressing to the legs or arms. In severe cases, loss of muscle tone in the hands and feet is also observed[8]. Neuropathic pain is diagnosed and treated differently than other types of chronic pain. This is the reason that makes the process more complicated and uncertain for the management of neuropathic pain. Neuropathic pain is linked to an increase in prescriptions for painkillers and also frequent visits to doctors[9] (Figure 1).

Figure 1. Symptoms of diabetic neuropathy.

Diabetic neuropathy is mainly classified into four types: peripheral diabetic neuropathy, autonomic neuropathy, proximal diabetic neuropathy and mononeuropathy. Peripheral neuropathy mainly reasons outside the brain and spinal cord i.e., legs and arms are affected, whereas autonomic neuropathy mainly affects the nerves of the organs of the autonomic system. It includes various body systems like sweat glands, eyes, bladder, digestive system and sex organs[10]. In proximal diabetic neuropathy, the nerves present in the thighs, hips, and buttock muscles are damaged. The cases of proximal diabetic neuropathy are more common in people with NIDDM and old aged people[11]. The last type is mono-neuropathy which is also called focal neuropathy. It includes damage to a single specific nerve which is observed often and may be observed in areas like arms, face, or legs[12]. Due to increased sensitivity caused by diabetic neuropathy, it has been found that diabetic individuals are more susceptible to nociceptive stimuli. *Clematis buchananiana* had been proven scientifically for hepatoprotective activity[13] (Figure 2).

Figure 2. Types of diabetic neuropathy.

The current research was done for screening other potent pharmacological actions of the plantsfor diabetic neuropathy. The study aims to unfold the neuroprotective action possessed by the plant so that it can be used in the future for the development of cost-effective herbal-based formulations that can be easily available in the market at low cost.

2. Materials and Methods

2.1 Animals

Adult Wistar male rats (8-12 weeks old, 150–200 g) were used for screening of the neuroprotective action of HEELCB. The animals were obtained from the Institute Animal House, NIET (Pharmacy Institute). All necessary guidelines from CPCSEA were followed while handling the animals from procurement till completion of the research activity. Animals were acclimatized for a period of 7 days under standard environmental conditions (temperature = 25 ± 2 °C; relative humidity = 35-60 % and 12 hr dark/light cycle). All animals were allowed free access to water *ad libitum* and a pellet diet. After the acclimatization period, the animals are divided into various groups as per the design of the experiment.

2.2 Drugs and Chemicals Used

Gabapentin as a standard drug, STZ as an inducing agent and acetic-acid solution as an inducing agent for pain was procured from CDH Limited, New Delhi, India. While glucometer from Accucheck was purchased from Kailash Surgical Chemists and Suppliers, Greater Noida, UP (India) for the determination of blood glucose levels in animals. All the solvents and chemicals were of analytical grade which was procured from CDH Pvt. Ltd. *Clematis buchananiana* leaves had been collected in October 2021 from the exterior of Dhanaulti, Uttarakhand. The taxonomic authentication of plant material was done by Dr Anjula Pandey, Scientist, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Furthermore, the leaves of *Clematis buchananiana* were put in the shade to dry before crushing them into a coarse powder. The ethanolic extract of coarse powder (leaves) was prepared and subjected to extraction through the soxhlet apparatus. The obtained ethanolic extract was concentrated in at water bath and dried.

2.3 Procurement of Drugs and Chemicals

The chemicals used during the experiment were of analytical grade. Gabapentin as a standard drug and acetic acid solution (0.6% v/v) was procured from Vitrag Pharma, Surat, India. A glucometer from Accucheck was used for the determination of blood glucose levels in animals. All the solvents and chemicals were of analytical grade which was procured from CDH Pvt. Ltd.

2.4 Acquisition and Preparation of Plant Sample

Clematis buchananiana leaves had been collected in October 2021 from the exterior of Dhanaulti, Uttarakhand. The taxonomic authentication of plant material was done by Dr Anjula Pandey, Scientist, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Furthermore, the leaves of *Clematis buchananiana* were put in the shade to dry before crushing them into a coarse powder. The ethanolic extract of coarse powder (leaves) was prepared and subjected to extraction through the soxhlet apparatus. The obtained ethanolic extract was concentrated in a water bath and dried.

3. Experimental Design

3.1 Acute Toxicity Test

Studies on the acute toxicity of chemicals i.e., acute oral toxicity tests were performed using healthy Wistar albino male rats sex weighing 120-180 gm under regular laboratory settings following OEC recommendations 423. After dosing, animals were watched daily for three days in a row, with at least one observation every 30 minutes for the first 30 minutes, and then every 24 hours after that (with a particular focus on the first 4 hours) (OECD, 423). A variety of measurements were taken daily to look for changes in the animal's skin and hair as well as eyes, nasal mucus membranes, respiratory rates, circulatory signals (heart rates), and autonomic effects (salivation, lacrimation, sweat, urine incontinence, and faeces). *C. buchananiana* was administered to six animals at a dosage of 2 gm/kg body weight and the animals' behaviour was monitored for 14 days to determine mortality and overall health. Until the completion of the research, none of the rats had died. Upto a dosage of 2 gm/kg body weight, the plant extract was deemed safe. Because of these findings, the safer doses which can be used for the evaluation of diabetic neuropathy are 100, 200, 300, 400, 500 and 600 mg/kg of the total body weight.

3.2 Induction of Diabetes Mellitus

A dose of 70 mg/kg was given by intravenous route in all the male Wistar albino rats of the five groups for inducing diabetes. The administration of streptozotocin by intravenous route induces diabetes within a time of nearly 72 hours[14]. For confirmation of diabetes, the rats in each group were tested for blood sugar by using a

diagnostic kit. After confirmation of diabetes, the animals were isolated and divided into groups for the development of neuropathy. Generally, neuropathy develops around the 14th-21st day in rodents[15]. Finally, after 21 days animals were assessed for neuroprotective effect by using experimental methods - tail immersion method and acetic-acid induced writhing method.

3.3 Induction of Diabetic Neuropathy

Diabetes was induced first. And after that hyperglycemic state was maintained for 12 weeks. Finally, animals were assessed for increased sensitivity and its effect on nerves to the estimation of the development of diabetic neuropathy[16]. The neuropathic development was assessed with the help of various parameters like locomotor tests and examination of the nerve tissue morphologically on weeks first, second, third, and fourth week in every group respectively.

3.4 Estimation of Fasting Blood Glucose

The rats in each group were kept on fast (overnight fasting i.e., 12 hours) and fed with water only before estimation of blood glucose level on preferred days 7th, 14th, 21st and 28th day respectively. The level of blood glucose was detected by withdrawing a drop of blood from the tail. Finally, a glucometer Accu-check was used for the detection of the amount of blood glucose level.

3.5 Screening of Neuroprotective Activity

The evaluation of neuroprotective action was done by using two standard animal models i.e., tail immersion method and acetic-acid writhing methods. The experimental methods and procedure are described separately below.

3.6 Tail Immersion Method

Water was maintained at a temperature of 55±0.5 °C and used in this experimental method for screening the neuroprotective effect of HEELCB extract in Wistar albino rats[17]. The experimental animals were divided into five different groups (n = 6) in each group. The first group (control) was fed and treated orally with saline, the second group standard was fed with gabapentin (100 mg/kg) and the remaining groups (Test 1, 2, and 3) were fed with HEELCB 100, 200, and 400 mg/kg respectively. In this method, 5cm from the lower portion of the tail was immersed in the beaker whose temperature was maintained at 55±0.5 °C and thermal hyperalgesia was induced. The percentage in response (jerking or withdrawal of the tail from the hot water) time was calculated after the specified period of 10s. The readings for all the experimental groups were taken at time intervals of 0 min, 15 min, 30 min, 60 min, and 90 min i.e., post-treatment. The calculations were based on various basal reaction times recorded at the time of experimentation.

3.7 Acetic Acid-Induced Writhing Method

For this study, all the selected animals were divided into five groups i.e., 6 rats in each group (n = 6). The first group (control) was fed and treated orally with saline (2 ml/kg); the second (standard) was fed with gabapentin (100 mg/kg) and the remaining groups (Test 1, 2 and 3) were fed with HEELCB 100, 200 and 400 mg/kg respectively. The determination of the analgesic effect was done with the help of a comparison between the significant reduction of writhes in tested animals compared to those in the control group and standard one[18]. Finally, % inhibition in pain was considered as an antinociceptive response (reducing sensitivity to painful stimuli) and was calculated with the help of the formula mentioned below:

$$\% \text{ Inhibition in Pain (Neuroprotective Effect)} = \frac{(N_C - N_T) * 100}{(N_C)}$$

Where, N_C = Number of writhing observed in the control group

Where N_T = Number of writhing observed in treated group (standard drug and test drug)

The number of reduced writhing observed for10 min after administration of acetic acid to the various groups by comparing with the control group.

4. Results

Table 1. Qualitative estimation of phytoconstituents in different solvents of leaves of *Clematis buchananiana*

Phytoconstituents	Solvents						
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ethanol	Distilled water	Hydroethanolic Extract
Alkaloids							
Dragendroff's Test	+	+	+	+	+	_	+
Mayer's Test	_	_	_	_	_	+	+
Wagner's Test	_	_	_	+	+	_	+
Hager's Test	+	+	+	+	+	+	+
Tannic acid test	+	+	+	+	+	+	+
Carbohydrates							
Molisch's test	+	_	_	_	_	_	-
Pentose test	_	+	_	_	_	_	-
Benedict's test	_	_	_	+	+	+	+
Tests for presence of Cardiac Glycosides							
Baljet's test	+	_	_	+	+	+	+
Legal's test	_	_	_	_	_	+	+
Steroids							
Libermann – Burchard test	+	_	+	_	_	_	+
Sulfur powder test	+	+	+	+	+	+	+
Flavonoids							
Shinoda test	_	_	_	+	+	+	+
Zinc hydrochloride	+	+	_	+	+	_	-
Saponin-glycoside	_						
Froth Formation test	+	_		+	+	_	+
Proteins	_						
Biuret test	_	_		+	+	_	+
Anthraquinone	_						
Hydroxy-anthraquinones	_	_	_	_	_	_	+
Amino acid- Millon's test	+	_		+	+	+	+
Tannins	+						
Ferric chloride test	_	_	+	_	_	_	+

(+) Indicate presence, (-) Indicate absence

The results of Table 1 reveals that hydroethanolic extract is found to possess the maximum number of phytoconstituents and hence it was concluded that it was the ideal solvent that can be used for extraction purpose as it contains a maximum number of phytoconstituents.

The results obtained after the end of acute toxicity studies reveal that hydroethanolic extract of leaves of *C. Buchananiana* in the range of 100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, and 1000 mg/kg is safer and can be used for evaluation of neuroprotective action.

The regular monitoring of blood glucose levels in different time intervals shows a persistent state of hyperglycemia. It means that the animals are ready for the conduction of the experiment.

Table 2. Outcomes of acute toxicity studies

S.N.	Treatment	Dose (mg/kg)				
		5	50	300	500	1000
1.	Control	0/3	0/3	0/3	0/3	0/3
2.	Standard	0/3	0/3	0/3	0/3	2/3
3.	Test 1	0/3	0/3	0/3	0/3	1/3
4.	Test 2	0/3	0/3	0/3	1/3	2/3
5.	Test 3	0/3	0/3	0/3	1/3	3/3

Table 3. Determination of fasting blood glucose

S.N.	Treatment	Fasting blood glucose level in mg/dl			
		Week 1	Week 2	Week 3	Week 4
1.	Control	95.4±0.18	96.35 ± 1.76	97.24 ± 2.04	97.03 ± 2.19
2.	Standard	154.01 ± 0.14*	153.13 ± 1.91*	141.32 ± 1.41*	121.18 ± 3.23*
3.	Test 1	152.14 ± 1.62*	159.37 ± 0.37*	149.38 ± 3.27*	129.01 ± 3.45*
4.	Test 2	152.53 ± 1.61*	152.16 ± 2.81*	135.14 ± 2.47*	117.12 ± 1.35*
5.	Test 3	152.79 ± 1.83*	152.46 ± 1.71*	123.16 ± 2.71*	115.18 ± 2.05*

Values are expressed as the mean ± SEM; n = 6. One-way ANOVA; followed by Dunnet's test; P < 0:05 in comparison with normal control and *P < 0:05 in comparison with control.

Table 4. Screening of neuroprotective action of HEELCB by tail immersion method

S.N.	Treatment and dose		Mean Latency (Sec) Before and After Drug Administration				
			0 min	15 min	30 min	60 min	90 min
1	Control (Saline 2 ml/kg)	Week 1	4.16±0.09	4.16±0.10	4.25±0.21	4.08±0.20	4.08±0.08
		Week 2	4.00±0.00	4.08±0.08	4.08±0.08	4.00±0.12	4.00±0.00
		Week 3	4.08±0.08	3.83±0.16	4.50±0.18	4.25±0.11	4.08±0.08
		Week 4	4.16±0.10	4.08±0.08	4.08±0.08	3.83±0.10	3.91±0.08
2	Standard (Gabapentin) (100 mg/kg)	Week 1	4.16±0.10 NS	3.83±0.10 NS	5.66±0.21**	6.00±0.25**	5.5±0.22**
		Week 2	4.16±0.16 NS	4.00±0.12 NS	5.91±0.23**	6.75±0.17**	5.75±0.17**
		Week 3	4.41±0.08 NS	4.16±0.16 NS	5.75±0.11**	6.41±0.15**	5.91±0.15**
		Week-4	4.41±0.08 NS	4.58±0.20	6.83±0.10**	7.66±0.16**	6.41±0.20**

Table 4 to be Continued...

S.N.	Treatment and dose		Mean Latency (Sec) Before and After Drug Administration				
			0 min	15 min	30 min	60 min	90 min
3	Test-I (HEELCB) (100 mg/kg)	Week 1	4.08±0.08 NS	3.91±0.15 NS	3.83±0.08 NS	3.83±0.16 NS	3.66±0.16 NS
		Week 2	4.00±0.16 NS	3.58±0.08 NS	3.66±0.16 S	3.58±0.08 NS	3.66±0.10 NS
		Week 3	4.16±0.16 NS	3.91±0.08 NS	4.33±0.10 NS	4.18±0.16 S	3.83±0.10 NS
		Week 4	4.25±0.11 NS	4.00±0.12 NS	4.41±0.08 NS	4.41±0.20 NS	5.41±0.10 NS
4	Test-II (HEELCB) (200 mg/kg)	Week 1	4.16±0.10 NS	3.91±0.08 NS	4.41±0.08 NS	4.33±0.21 NS	3.83±0.10 NS
		Week-2	4.16±0.16 NS	4.08±0.15 NS	4.41±0.15 NS	4.25±0.11 NS	4.33±0.10 NS
		Week 3	4.33±0.10 NS	3.91±0.08 NS	5.16±0.10**	5.16±0.10**	4.66±0.10**
		Week 4	4.25±0.11 NS	4.25±0.11 NS	5.41±0.15**	5.66±0.16**	5.41±0.08**
5	Test-III (HEELCB) (400 mg/kg)	Week 1	4.16±0.10 NS	4.00±0.12 NS	4.66±0.10 NS	4.66±0.10 NS	4.16±0.10 NS
		Week 2	4.16±0.10 NS	4.08±0.08	4.25±0.11 NS	4.33±0.10 NS	4.25±0.11 NS
		Week 3	4.33±0.10 NS	4.25±0.11 NS	5.66±0.21**	5.75±0.11**	4.88±0.10**
		Week 4	4.41±0.08 NS	4.58±0.15 NS	6.00±0.12**	6.83±0.16**	5.66±0.10**

The values are represented as mean ± standard error of the mean (SEM). Statistical significance was analyzed by way of ANOVA with Dunnett's T-test. P values of < 0.05 were considered statistically significant.

The tail immersion method used for evaluating the neuroprotective action of hydroethanolic extract of leaves of *C. buchananiana* shows that neuropathy is a long-term disorder associated with nerve damage. The repair and healing time is longer in duration. It also reveals that after 21 days there was an increase in the threshold value of pain, which indicates that the plant is effective and possesses neuroprotective action. The maximum basal reaction time was observed at the end of the fourth week. The efficacy order of the various doses used of HEELCB in decreasing series is 100 mg/kg (standard drug - Gabapentin), 400 mg/kg (test drug-III), and 200 mg/kg (Test drug-II).

Table 5. Screening of neuroprotective action of HEELCB by acetic acid method

S.N.	Drugs/Dose	Time	No. of Writhing	% Inhibition (Neuroprotective Action)
1.	Control (2 ml/kg)	Week-1	58.16±0.90	NA
		Week-2	56.66±1.05	NA
		Week-3	55.00±0.00	NA
		Week-4	55.00±0.00	NA
2.	Standard (Gabapentin) (100 mg/kg)	Week-1	50.00±0.00**	14.28
		Week-2	43.66±0.88**	22.49
		Week-3	36.50±0.84**	33.63
		Week-4	28.00±0.77**	48.96
3.	Test-I (HEELCB) (100 mg/kg)	Week-1	57.16±0.47 NS	1.72
		Week-2	55.33±0.21 NS	1.77
		Week-3	53.00±0.85 NS	3.48
		Week-4	53.66±0.21 NS	2.43

Table 5 continued...

S.N.	Drugs/Dose	Time	No. of Writhing	% Inhibition (Neuroprotective Action)
4.	Test-II (HEELCB) (400 mg/kg)	Week-1	55.60±0.49 NS	4.41
		Week-2	56.33±0.88 NS	0.58
		Week-3	43.83±0.70**	20.30
		Week-4	40.83±0.40 **	25.76
5.	Test-III (HEELCB) (400 mg/kg)	Week-1	56.66±1.05 NS	2.58
		Week-2	52.50±0.84 NS	7.34
		Week-3	43.33±0.55**	21.21
		Week-4	36.50±0.56**	33.63

The values are represented as mean ± standard error of the mean (SEM). Statistical significance was analyzed by way of ANOVA with Dunnett's test. P values of < 0.05 were considered statistically significant.

The acetic-acid model of pain employed for estimating the neuroprotective action of hydroethanolic extract of leaves of *C. buchananiana* shows a significant decrease in the number of writhings produced in all the experimental animals. The positive and pharmacological effect was observed after 21 days and the maximum effect after 28 days. The efficacy order of the various doses used of HEELCB in decreasing series is 100 mg/kg (standard drug- gabapentin), 400 mg/kg (test drug-III), and 200 mg/kg (test drug-II).

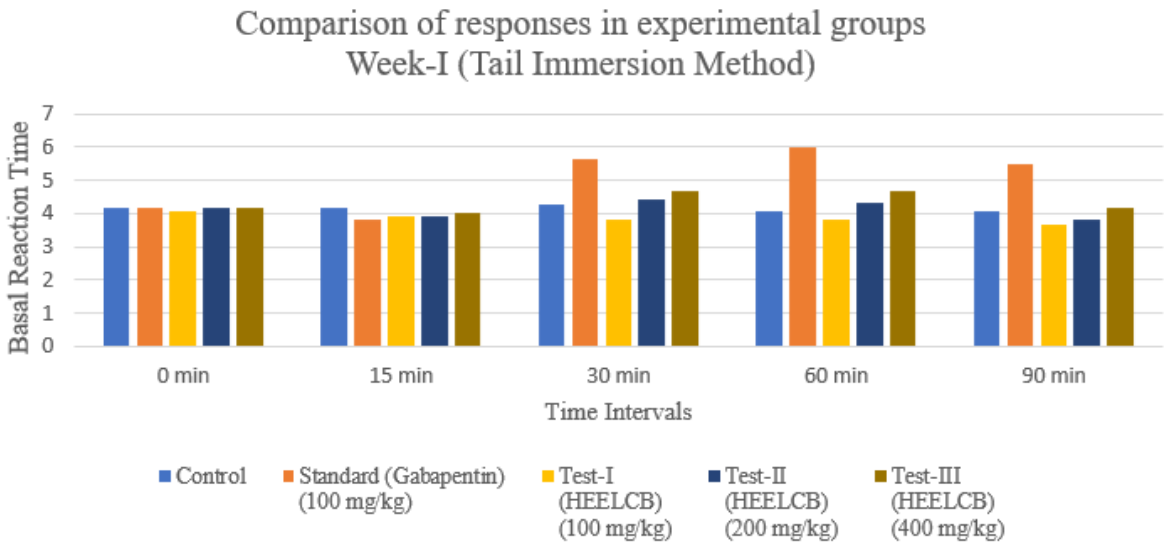

Figure 3. Screening of neuroprotective action of HEELCB by tail immersion method for week-I.

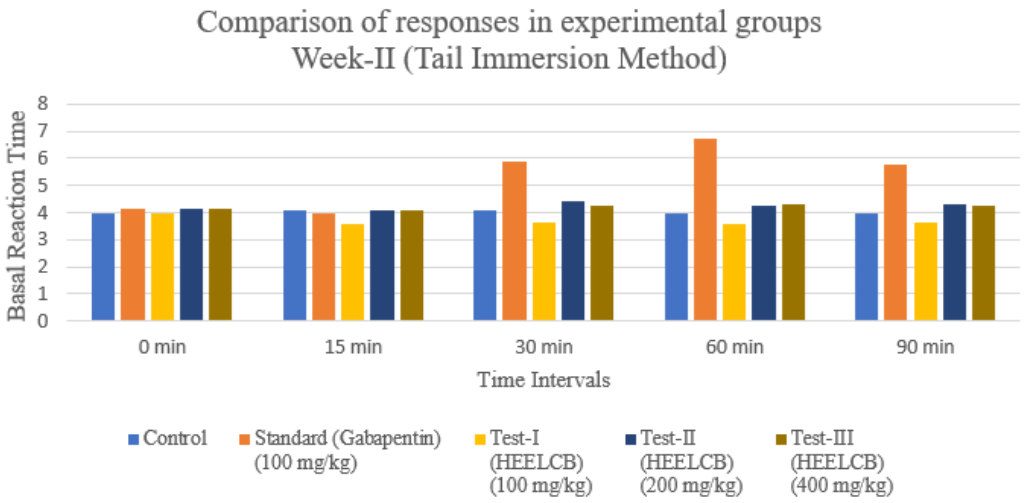

Figure 4. Screening of neuroprotective action of HEELCB by tail immersion method for week-II.

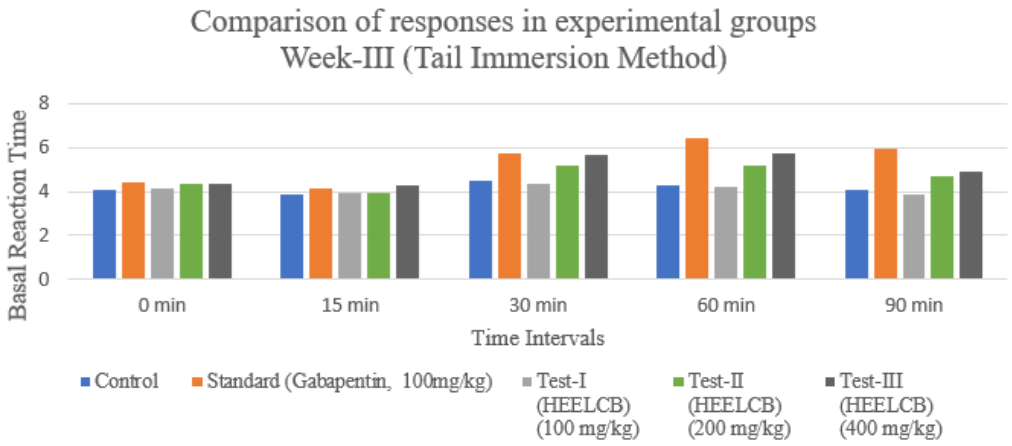

Figure 5. Screening of neuroprotective action of HEELCB by tail immersion method for week-III.

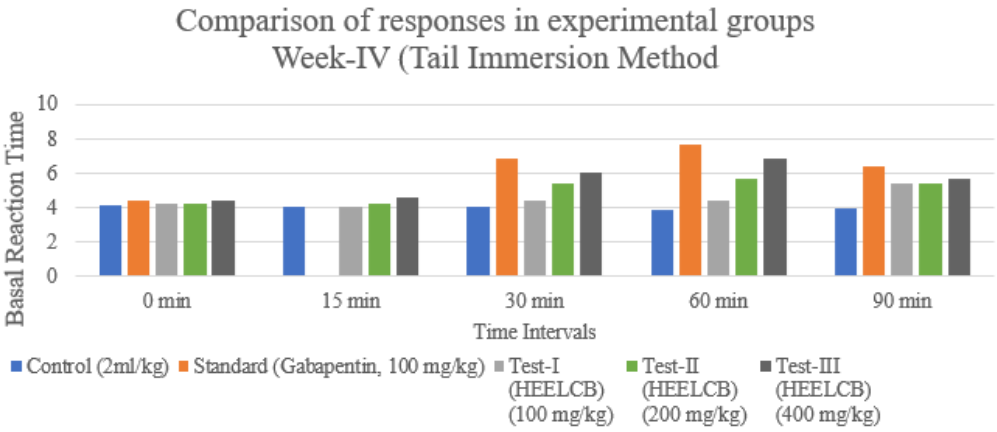

Figure 6. Screening of neuroprotective action of HEELCB by tail immersion method for week-IV.

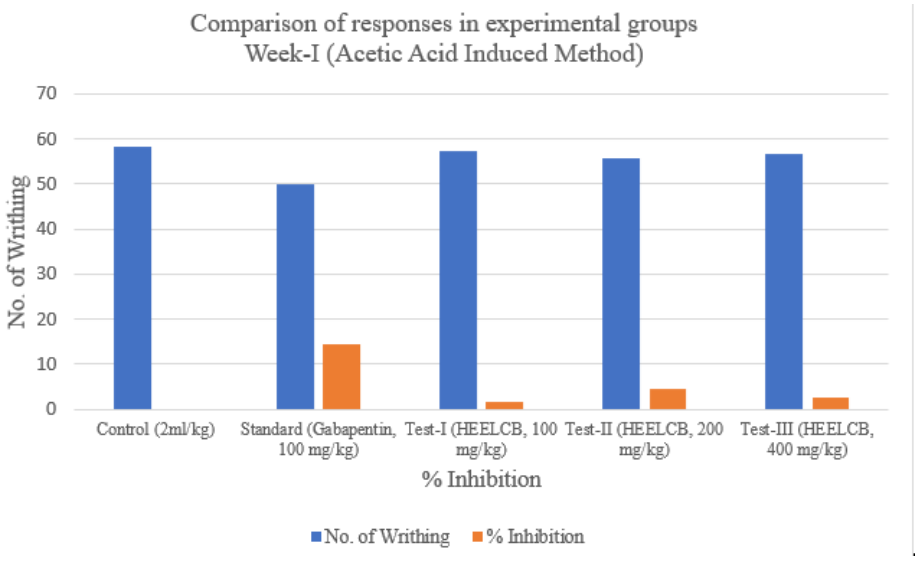

Figure 7. Screening of neuroprotective action of HEELCB by acetic acid method week-I.

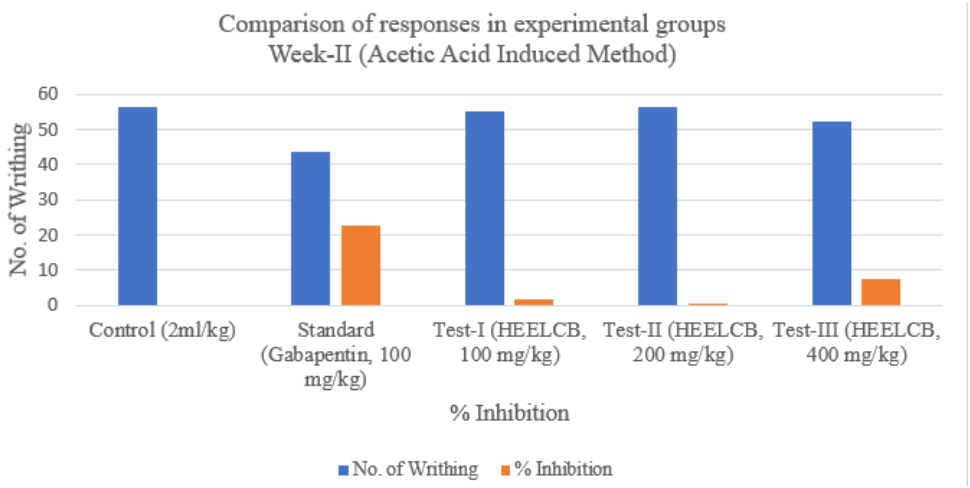

Figure 8. Screening of neuroprotective action of HEELCB by acetic acid method week-II.

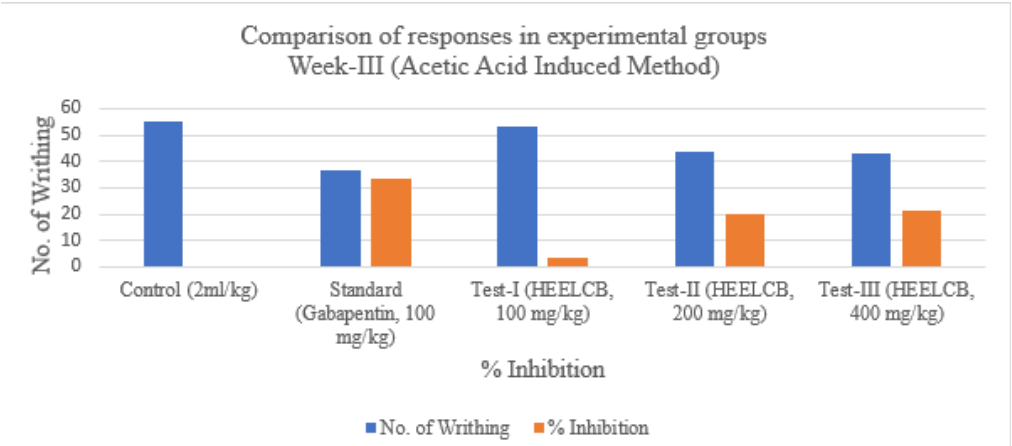

Figure 9. Screening of neuroprotective action of HEELCB by acetic acid method week-III.

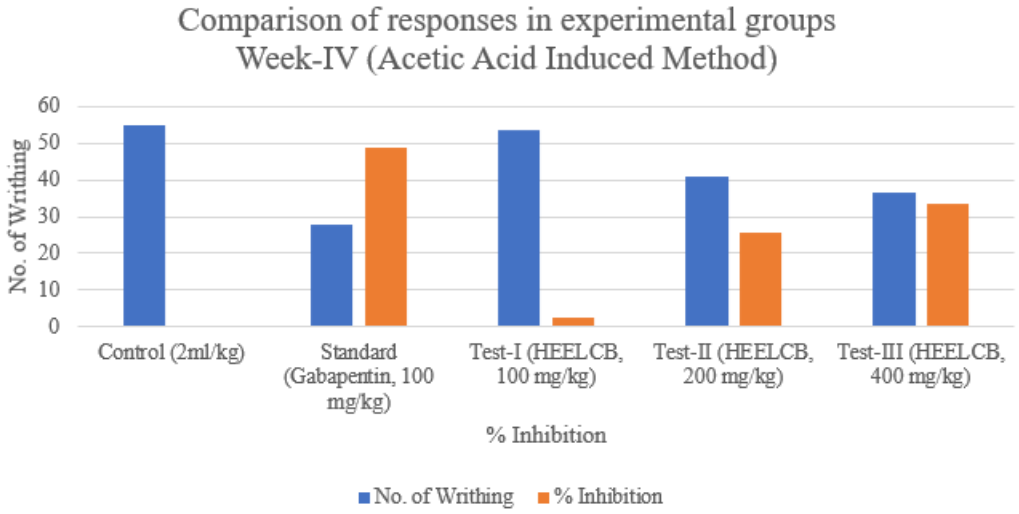

Figure 10. Screening of neuroprotective action of HEELCB by acetic acid method week-IV.

5. Discussion

5.1 Acute-Toxicity Test

The animals were under continuous and minute observation for 14 days after administration of the various doses of the test drug HEELCB. For confirmation of toxicity, essential parameters like the presence of any unintended sign on skin, hair, eyes, irritation in the mucous membrane, respiratory signs like difficulty in breathing/shallow breathing, and other vital parameters like change in autonomic reflexes like increased lacrimation, salivation, urine, faeces, etc. were observed as per the guidelines. After 14 days, the animals were sacrificed and vital organs (like liver, brain, etc.) were isolated for determination of the presence of any signs of toxicity. The results are shown in Table 2.

5.2 Fasting Blood Glucose

The blood glucose levels were measured in all the experimental groups (which had been shown in Table 3). The results obtained met the required level of blood glucose level in animals and were sufficient for the development of diabetic neuropathy. The groups mainly: Group-III, Group-IV, and Group-V reported blood glucose levels in the ranges: 129.01 ± 3.45, 117.12 ± 1.35, and 115.18 ± 2.05 mg/dl concentrations respectively. There was not any significant level of improvement in the blood glucose level of the experimental animals when compared with the control group on the final day 28th day of the experiment. While Group-II showed some level of improvement in blood glucose levels with 121.18 ± 3.23 mg/dl concentration on the 28th day.

5.3 Estimation of Neuroprotective Action by Using Tail Immersion Method

The results shown by hydroethanolic extract of the leaf of *Clematis buchananiana* (HEELCB) were quite significant. The increase in basal reaction time for the Tail immersion method (the withdrawal of the tail from the source i.e., tail latency) was found 14 days after the induction of diabetic neuropathy. The test groups treated with HEELCB i.e., the experimental Group-IV and Group-V when compared with the control Group-I showed a significant result. The experimental data reveal that the treatment with the standard drug Gabapentin in Group-II (Dose: 100 mg/kg) showed improvement in symptoms since the first week of administration of the drug. Whereas Test Group-III (Dose: 100 mg/kg) did not show any significant results after the completion of 28 days also. The results were found to be statistically significant ($p<0.01$).

The current data obtained, suggests that the plant is rich in phytoconstituents like alkaloids, triterpenoids, tannins, flavonoids, glycosides, etc. Hence, the plant can be investigated further for bioassay methods for unfolding the hidden pharmacological potency of the plant. The experimental results showed a significant decrease in pain

which means that the plant possesses neuroprotective action (the neuroprotective action was screened by animal models described in the research methodology). The extract i.e., HEELCB at a dose of 200 mg/kg and 400 mg/kg showed a significant reduction in tail latency as compared to that of the control group of rats. Whereas the standard group, Gabapentin treated (100 mg/kg) exhibited the maximum analgesic effect (neuroprotective effect) effect in between the 3rd week and 4th week. It showed significant ($P<0.05$) improvement in tail withdrawal latency (assessed by tail immersion method. While in the acetic acid-induced writhing method significant level of % Inhibition (neuroprotective effect) was observed. All data were subjected to ANOVA followed by Dunnett's test, the observation was Mean±SEM. *$P<0.05$ as compared to the normal control group, standard group, and $P<0.05$ as compared to the diabetic group of the test compound. The results are tabulated in Table 4 (Figures 3-6).

5.4 Estimation of Neuroprotective Action in Rats by Acetic-Acid-Induced Writing Method

For % inhibition in pain (neuroprotective action) Wistar albino rats were used as experimental animals. For determining the % inhibition of the experimental animals, animals were previously treated with standard drugs and various doses of HEELCB. Animals were divided into five groups Group-I, control (2 ml/kg normal saline), Group-II, Standard (Gabapentin:100 mg/kg), Group-III, Test-I (HEELCB: 100 mg/kg), Group-IV, Test-II (HEELCB: 200 mg/kg) and Group-V, Test-III (HELCB: 400 mg/kg) respectively. Each animal was administered prescribed doses of the test and standard drug. After 30 min 0.6ml v/v acetic acid was injected into each group. Finally, the number of writhing was observed for 10 min. The same process was repeated for four weeks till results were not obtained. The analgesic response (neuroprotective action) was calculated by using stats. During the research study animals were fed once daily for 28 days daily before taking responses. The Group-I Control (Normal Saline Treated) did not show significant % inhibition in pain after completion of the research activity. Whereas the Group-II Standard (Gabapentin: 100 mg/kg) exhibited analgesic action since the administration of the drug from Week-I and the % inhibition in pain increased significantly from Week-I till Week-IV (maximum % inhibition was observed in Week-IV). Whereas, Group-III, Test-I (HEELCB: 100 mg/kg) did not show any significant effect when compared with the Control group after all week's completion. But the results in Group-IV i.e., Test-II (HEELCB: 200 mg/kg) showed significant responses after the 3rd and 4th week when compared with Group-I control. The significant % inhibition in pain (neuroprotective action) for the test compound was observed for the higher doses of HEELCB i.e., Group-V (HEELCB: 400 mg/kg) followed by Group-IV i.e., Test-III (HEELCB: 200 mg/kg). The results had been described in Table 5 (Figures 7-10).

6. Conclusion

After the end of the research study, it was found that the hydroethanolic extract of the leaf of *Clematis buchananiana* showed improvement in timing in pain whereas the number of writings also decreased. Both methods show that the plant is having neuroprotective action which was observed as analgesic action (the property of relieving pain from noxious stimuli leading to pain). The experimental results obtained during the research work gave significant results. The basal reaction time increased and % inhibition in pain increased (neuroprotective action achieved) after continuous treatment in each of the animals of the five groups except Group-I, i.e., Control group and Group-III (TEST-I, 100 mg/kg HEELCB). The study unfolds the pharmacological property in the form of neuroprotection and analgesic action in Wistar albino rats suffering from diabetic neuropathy. The damaged nerves due to hyperglycemia cannot be reversed completely but it can be surely repaired on a cellular level. *Clematis buchananiana* species had been unreported to other pharmacological properties like antioxidant and hepatoprotective. Since herbal-based formulations are in greater demand these days, the development of cost-effective herbal-based formulations for the treatment of vulnerable diseases with limited treatment can be utilized for the maintenance of better health in current and future generations.

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8. References

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